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Reference Intervals for Haematological and Morphometric Parameters in Balkan Donkey in Serbia

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Abstract

The Balkan donkey is an indigenous primitive breed that is bred in the hilly and mountainous regions of Serbia and Montenegro. It represents an important resource in terms of preserving the genotype of our country. Today, the majority of donkey population in Serbia is situated in Special Nature Reserve "Zasavica", Nature park of "Stara Planina and Kovilj vilage, near Novi sad. They are mainly used for the production of donkey milk and as a tourist attraction on farms. There are no many literature data related to of the values of basic hematological and morphometric parameters in donkeys. This led to the development of a this study, especially on donkeys from Stara Planina, whose end result were the definition of basic physiological values of triassic parameters, hematological and biochemical values of blood parameters and precise morphometric measurements. Knowing the values of these parameters is of great importance for veterinarians for the correct interpretation of clinical findings and diagnosis of diseases in donkeys.

Keywords: Balkan donkey; blood; hematological; morphometrical parameters; Triassic.

1. Introduction

According to literature data, there are 163 breeds of donkeys in the world, that amounts about 44 million individuals [22]. In Europe and developed countries, population of these animals is constantly decreasing, while some breeds around the world are pre-extinction.

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The Balkan donkey is an autochthonous, primitive breed that inhabits the mountainous regions of Serbia and Montenegro [24].These donkeys were mainly used as pack animals that transported the load from the mountain pastures to the settlements in the valleys, and then for pulling carts or plows and riding. However, the development of agriculture and the departure of the human population from mountain villages, especially during the second half of the last century, led to a sharp decline in the number of Balkan donkeys [7].

As an important resource regarding the preservation of genotypes in our country, preservation of Balkan donkey is in the program for the conservation of animal genetic resources and the state has introduced incentives for owners to keep them. That contributed to the renewal of the reproductive potential of these individuals. In 2022. officials records an increase in their number. According to the available DID-IS (Domestic Animal Diversity Information Service) FAO data for 2024, the number population of Balkan donkeys amounts about 500-1000 specimens and currently they have the status of an endangered maintained breed [14]. Today, the largest part of the population of these donkeys in Serbia can be found in 3 locations: in the special nature reserve "Zasavica", the national park "Stara planina" and in the village of Kovilj, not far from Novi Sad .

In the area of the "Stara planina" national park, there are about 250 donkeys that are under direct observation of the Teaching Base for Mountain Animal Husbandry of the Faculty of Veterinary Medicine of the University of Belgrade. All these animals are kept on pastures most of the year and are used for milk production and as a tourist attraction on the farm. Donkey's milk is of higher quality than cow's milk, which is why it is widely used by humans [18]. Also, thanks to the low content of saturated and high the content of unsaturated fatty acids, the consumption of donkey milk has important role in the therapy and prevention of cardiovascular and autoimmune diseases [2,18].

In the literature, there were no data on basic values of physiological and morphometric parameters in Balkan donkeys, so the values obtained from these animals by veterinarians are often compared with values for horses or values of other donkey breeds. This led to this study, especially on donkeys from the Stara planina area, whose task was to define the normal values of basic physiological and morphometric parameters. [1,8,11]. Knowing these values is of great importance for clinical diagnostics and future tests on this population of animals.

2. Materials and methods

A total of 18 adult Balkan donkeys: 10 females and 8 males, aged between 4 and 16 years, weighting 150-250 kg were included in the study. The females were bred for production of the donkey milk. Animals were identified upon microchip number, coat color, distinctive signs, and age of each animal was determined upon dentition. The study was performed during annual veterinary health assessment in accord to the Animal Health Regulations of Republic of Serbia. Donkeys were in good condition (BCS -body condition score) ranged from 4 to 6, and physically healthy. During the night donkeys were confined in a compartmentalized stable (winter shelter) and free grazing during the day, with addition of common forage diet while in confinement [10]. All animals included in the study were thoroughly clinically examined before the evaluation as only fully grown and sound animal could be used for measurements.

2.1 Haematolgical and morphometric measurements

1. Blood was sampled from clinically healthy donkeys by vein punction of the jugular vein,. The stress was minimized by handling the animals with care before the collection of samples. The blood was collected in EDTA tubes for hematological studies, and into vacutainer tubes with coagulation stimulation for serum extraction. Immediately after sampling, the tubes were chilled on ice. Hematological profile and serum separation were performed within 2h following the blood sampling. Plasma and serum were harvested by centrifugation at 2.800 x g for 5 minutes. Complete blood counts with related parameters were determined with an automatic cell counter (Abacus Junior Vet, Diatron), using the pre-formatted software for analyzing horse's blood. The following parameters were included in the hematological analysis: white blood cells (WBC), lymphocytes (LYM), mid cells count (MID), granulocytes (GRA), red blood cells (RBC), hemoglobin (HGB), mean cellular hemoglobin (MCH), mean cellular volume (MCV), platelet cells (PLT), mean platelet volume (MPV), hematocrit (HCT), mean cellular hemoglobin concentration (MCHC) and platelet count (PCT). The biochemical profiles were obtained by using a semi-automated biochemistry analyzer (Vet Evolution, BSI) with the appropriate reagent kits, in accordance with the recommended standard protocols. The biochemical panel included the following parameters: aspartate aminotransferase (AST), alkaline phosphatase (AP), urea, creatinine, albumin, total protein, lactate dehydrogenase (LDH), creatine kinase, γ-glutamyltransferase (GGT), total bilirubin, cholesterol, triglyceride, calcium (Ca) and inorganic phosphorus (P).

The following morphometric parameters were measured: withers height (WH), body length (BL), chest length (TL), thoracic perimeter (TP), thoracic depth (TD), chest width (CW) and cannon circumference (CC) were estimated by tape measuring in aim to reduce manipulation stress and body weight (BW) was estimated upon the formula (1) by Pejić [16]:

BW (kg) = (TP2 x BL) / 11877 (1)

Body frame was determined as ratio of WH to BL multiplied by 100, relative body index as ratio of BL to TP multiplied by 100, while Conformation index (CI) was determined upon the formula (2) [13]:

3. Results

3.1 Triassic parameters

Our results correspond to expected values of triasic parameters recorded in other studies, which examined the physiological values of certain clinical and laboratory parameters in domestic mountain horses and several donkey breeds. Stara planina and indicated the values of reference basic vital -Triassic parameters in of these animals [3, 5, 6, 8] The results of this study are shown in Table 1.

The average heart rate values of the Balkan donkey 45.76 /min, temperature 36.18 ^{o}C and breathing rate 23.29 /min. Also, there are differences in the values of temperature and respiration between these two animal species, and that the values of temperature are higher in horses, in contrast to the breathing frequency which is higher in donkeys [3,4,8,19].

Table 1: Values of Triassic parameters in horses and donkeys

Parametar	Horses		Donkeys	
	Mean value ± SD	Range	Mean value ± SD	Range
Heart rate (/min)	43,66±7,96	32-56	45,76±6.62	32-52
Temperature (ºC)	37,61±0,14	37,4-37,8	36,18±0,47	35,4-36,8
Respiration(/min)	13,16±5,60	8-24	23,29±3,23	20-28

3.2 Hematological and biochemical blood parameters

2. In our study we obtained results that show similarity to values of other donkey breeds. Knowledge of reference values of hematological and biochemical parameters is an important diagnostic tool in veterinary clinical practice that should enable the clinician to gain insight into the patient's health condition more easily. Values of these parameters can be influenced by gender, race, age, and other factors like environment, diet, housing conditions, as well as the method of material sampling and laboratory errors during processing [4, 20, 23].

Table 2: Reference hematological values of donkeys from Stara planina,

Parameter	Unit	Mean	± SD	Range
RBC	$x10^{12}$/l	5,28	0,58	4,58–6,37
Le	$x10^{12}$/l	20,71	3,32	16,97–26,61
Gr	$x10^{12}$/l	13,13	1,35	11,59–15,22
Li	$x10^{12}$/l	7,13	2,93	2,37–11,74
Hct	l/l	31,35	3,32	27,38–38,17
Hgb	g/l	104,33	10,14	96–122
MCV	fl	59,55	4,15	53–65
MCH	pg	19,82	0,72	18,8–20,9
MCHC	g/l	333,55	13,24	312–352
RDWc	%	21,45	1,09	20,5–23
Plt	$x10^{9}$/l	208,77	64,01	94-291

If we compare our results to other studies, we found higher values of red blood cells in donkeys in the "Zasavica" special nature reserve and lower leukocyte cell values compared to donkeys from Stara Planina [28]. In other study on the population of Balkan donkeys on Stara planina, researchers report results similar to these. [8]. Values for MCH and MCHC were decreased, while platelet count values were elevated. That might be explained in better forage condition in Zasavica compared to Stara Planina.

Establishment of reference hematological values of Balkan donkeys at Stara Planina prompted the researchers to conduct the study on the same population of animals with the aim of determining the values of basic biochemical parameters [23]. The results of this study are presented in Table 3. Authors report that the values for ALP activity, AST and urea and creatinine concentration in Balkan donkeys are much lower compared to the same values of donkeys bred in Ethiopia [19]. Also, by comparing the value- comparison of the biochemical parameters of the Balkan donkey with the reference values of the biochemical parameters of donkeys in Brazil [13] confirmed reduced values of AST, LDH activity and urea and creatinine concentrations. l parameters of the Balkan donkey with the reference values of the biochemical parameters of donkeys in Brazil [13] confirmed reduced values of AST, LDH activity and urea and creatinine concentrations.

Table 3: Reference results of biochemical tests of individual biochemical values parameter in Balkan donkeys on Stara planina

Parameter	Mean	± SD	SEM	Range
AST (IU/l)	148,6	61,0	19,3	78,0-277,0
ALP (IU/l)	302,0	59,9	18,9	192,0-374,0
GGT (IU/l)	41,1	15,9	5,0	14,8-66,0
CK (IU/l)	114,4	72,3	22,9	59,0-306,0
LDH (IU/l)	420,3	86,9	27,5	324,0-554,0
Urea (mmol/l)	6,9	0,7	0,2	5,9-8,1
Kreatinin (μ mol/l)	59,9	18,1	5,7	41,5-105,0
Ukupni bilirubin (μmol/l)	6,9	1,8	0,6	4,6-9,6
Holesterol (mmol/l)	1,8	0,3	0,1	1,5-2,4
Trigliceridi(mmol/l)	2,6	0,9	0,3	0,9-4,0
Ca (mmol/l)	2,9	0,3	0,1	2,3-3,0
P (mmol/l)	1,2	0,2	0,1	1,0-1,7
Total protein (g/ l)	79,4	5,3	1,7	68,0-88,0
Albumini (g/l)	30,2	1,9	0,6	28,0-33,5

3.3 Morphometric measurments

The morphometric data obtained were used to establish the data in relation to development of the body, i.e. body

frame, thoracic index and conformation index in Balkan donkey in Serbia. Upon the calculated body indexes (Tabe 4.) it was concluded that frame of the donkeys can be slightly rectangular, or almost square, and that thorax is elongated, proportionally deep (sternum reaching the elbow) but narrow in all animal included.

The morphometric parameters established were used for determination of body indexes that reflect the development of animal, and allow description of the shape, size and volume of body. In comparison of body measurements established with literature data, we found that tested parameters in the Balkan donkey are similar to donkey breeds from neighboring countries and differ from large donkey breeds like Spanish Zamorano Leones donkey [3, 6, 12,17]. Papa and his colleagues [5,15] evaluated morphometric characteristics of donkey populations in Albania in three regions: lowland, upland and mountain. Their results show similarities in height between the lowland donkey (115,3 ± 8,2cm) and our indigenous breed [21]. In Albania lowland donkey type is defined as a standard type, while highland/mountain type is classified as a miniature Mediterranean type of donkeys. Another study which was done on donkey population in some regions in Bulgaria, shows morphometric congruence between the height of the mountain donkey (112.94±7.21cm) and the results of our study [26, 27]. Morphometrical studies in Zamorano-Leones donkey, a Spanish breed, approximated the WH value at 140cm-155cm [9,11, 17,], which, compared to our results, shows that the Zamorano-Leones donkey is significantly higher than Balkan donkey in Serbia. Furthermore, Zamorano-Leones has larger thoracic cage and is heavier than Balkan donkey 131-235kg to 245-330kg. The established biometric data reveal characteristic rectangular body frame with narrow chest in Balkan donkey in Serbia, which is similar to already published morphometric data [25,28].

Table 4: Morphometric measurements for the Balkan donkey breed (mean ± standard deviation; range for the whole group)

Parameter	Range (Min -Max)	Mean + SD
Withers height (cm)	93 - 129	109.75±11.34
Body length (cm	88 - 131	115±11. 64
Chest length (cm)	48 - 64	54.17±4.84
Thoracic perimeter (cm)	107 - 134	118.25±9.64
Thoracic depth (cm)	56 - 80	65.92±6.11
Chest width (cm)	22 - 26	23.25±2.26
Cannon circumference (cm)	10 - 15	12.41±1.98
Body weight (kg)	131- 235	174.5±36. 92
Frame index (cm)	90.16 -105.68	95.55±4.54
Chest index (cm)	27.14 - 42.86	35.5±4.36
Conformation index (cm)	109.39 - 140.54	121.22±9.33

4. Conclusion

Knowing the normal values of the basic physiological parameters and morphometric mesurments in the Balkan donkey is of great importance for the correct housing, selection and interpretation of clinical parameters findings and diagnosis of diseases in these animals. Additional tests on a larger number of individuals are needed to form a complete database for all values of clinical and laboratory parameters that will help with better understanding of this autochtonous breed.

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